

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20-986

PHARMACOLOGY REVIEW(S)

MAR - 8 2000

NDA 20-986

Review completed: March 6, 2000

Sponsor: Novo Nordisk Pharmaceuticals Inc., 100 Overlook Center, Princeton, NJ.

Date Submitted: November 5, 1999 (NDA Amendment For Labeling), 11/30/99, and 12/6/99.

Date Received: November 8, 1999.

Drug Class: Insulin Aspart (Insulin X-14, _____), L-Aspartic acid-insulin human (Recombinant human insulin, DNA origin, β^{28} Asp-Insulin).

Category: Insulin analog

Indication: Treatment of diabetes (type I and 2).

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/S/
Indra Antonipillai, Ph.D.

cc:

NDA Arch
HFD-510
HFD-510/steigerwalt/antonipillai/koller/jrhee
X14 insulin analog

Concur
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3/8/00

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ON ORIGINAL

**REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA FOR NDA
LABELING AMENDMENT**

KEY WORDS: Insulin, diabetes, glucose

Reviewer Name: Indra Antonipillai

Division Name: Division of Metabolic and Endocrine Drug products.

HFD# 510

Review Completion Date: January 31, 1999

IND/NDA number: NDA 20-986

Serial number/date/type of submission: November 5, 1999, NDA labeling amendment

Information to Sponsor: Yes (x) No () - (labeling)

Sponsor or agent: Novo Nordisk Pharmaceuticals Inc., 100 Overlook Center, Princeton, NJ.

Manufacturer (if different) for drug substance: Novo Nordisk A/S, Novo Alle, DK-2880 Bagsvaerd, Denmark.

Drug:

Code Name: Insulin Aspart (Insulin X-14 _____)

Generic Name: N/A

Trade Name: N/A

Chemical Name: L-Aspartic acid-insulin human (Recombinant human insulin, DNA origin, β^{28} Asp-Insulin))

CAS Registry Number (if provided by sponsor): N/A

Molecular Formula/ Molecular Weight: $C_{256}H_{381}N_{65}O_{79}S_6/5825.8$

Structure:

Drug Class: Insulin Analogue

Indication: Treatment of diabetes (type I and 2)

Route of administration: subcutaneously in the abdominal wall, the thigh, or the upper arm.

Proposed clinical protocol or use: Insulin aspart is indicated for the treatment of diabetes mellitus as a rapid acting insulin analog. It should be taken immediately before meal. _____ . It should be used in regimens together with intermediate or long-acting insulin. The physician, depending on the needs of the patient determines dosage, and it should be regularly adjusted according to blood glucose measurements. The individual insulin aspart requirement is usually between 0.5-1 U/kg/day, _____

LABELING

1. **Carcinogenicity labeling:** The sponsor has proposed the new text for the label, based on following issues:
 - a. NovoLog significantly increased the incidence of mammary tumors, but it was only seen at a high dose of 200 U/kg/day in a paired t test ($p=0.004$) or peto's analysis ($p=0.003$, a trend test) in a QA study (#940301, T12 study). Our statistician agrees, (the statistician's review is included in the appendix) that in a trend test when the group treated with high dose was excluded, the trend test at mid-low doses (10-50 U/kg/day of Novolog) was not significant ($p=0.21$).
 - b. The drug did not increase the tumors above insulin in T12 study (all tumors $p=0.062$, NS). Our statistician again agrees that this was not significant in a pairwise comparison (at 0.05 significance level).
 - c. In a non-QA study (#930803, T13 study), this difference between insulin and NovoLog was not seen, and only insulin at 200 U/kg/day increased the incidence of benign tumors, but not NovoLog .
 - d. The sponsor has performed the combined statistical analysis on two-studies (a non-QA report, and a QA study), and p for tumorigenicity of insulin vs NovoLog was not significant ($p=0.29$).
 - e. They also quote another study (# 940267) where human insulin at 150 U/kg/day produced significant malignant tumors ($p=0.009$), as well as all tumors ($p=0.002$).
 - f. They state that tumorigenicity is probably linked to mitogenic action of insulin/IGF-1 receptor, and their preclinical data state that NovoLog is equivalent to insulin (in terms of insulin and IGF-1 receptor affinity, mitogenic potency, and insulin receptor dissociation rate). Therefore, it is equivalent to insulin in growth promoting activity. It is true that the affinity of X14 for the insulin receptor is similar to that of human insulin (92% with X14, vs 100% with human insulin), and its affinity for the IGF-1 receptor is slightly higher than human insulin (0.05% with X14, 0.03% with human insulin, vs 100 % with IGF, _____). _____ had 7-fold higher affinity to IGF-1 than _____ X14 had 2-fold higher affinity than insulin. The mitogenicity studies were inconclusive.
 - g. In clinical trials with NovoLog, there are no reports of cancer after exposure of 2011 subjects for 1434 treatment years (F 661, M 773 years).
 - h. Based on U/kg/day, no tumor effects of insulin or NovoLog were seen up to 50-60 U/kg/day (corresponding to 50-fold the human dose based on U/kg/day, or plasma insulin levels). These are only seen at 150-200 fold the human dose. The sponsor claims that this high dose (150-200 U/kg/day) produced 25-56% mortalities within 1-year, which questions the experimental design of the study. Therefore, mammary tumors observed at extreme doses in a QA study are of not clinical significance. However in a QA study, more deaths occurred with insulin than analog (both at high doses of 200 U/kg/day).

[]

We presented the new label to Executive Carcinogenicity committee, based on the issues that the sponsor had raised. The package submitted to the Executive CAC Committee, along with their recommendations is presented below.

2. **Pregnancy labeling**: The sponsor mostly agrees with us on the recommended label, but would like to still call it pregnancy category — The sponsor argues the label on following issues

[

_____ They want it stated that "these effects are probably secondary to maternal hypoglycemia at high doses".]

Again, we took this issue up with the Reproductive Toxicity Assessment Committee (based on the supporting arguments provided by the sponsor). We had suggested category — to the Reproductive Tox Committee, and indicated not to penalize the sponsor for testing higher doses. The package submitted to the Reproductive Tox Committee, along with their recommendations is also appended here. However, based on findings with the drug (novolog), the Committee recommended a category 'C'.

Proposed New Label Text by the sponsor on 11/5/99 amendment:

"Carcinogenicity, Mutagenicity and Impairment of Fertility

_____ have not been performed to evaluate the carcinogenic potential of NovoLog™. In 52-week studies with Sprague-Dawley rats dosed subcutaneously with NovoLog™ _____

_____. NovoLog™ was not genotoxic in the following _____ tests; Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, in vivo micronucleus test in mice, and in ex vivo UDS test

in rat liver hepatocytes. In fertility studies in male and female rats at subcutaneous doses up to 200 U/kg/day (approximately 32 times the human subcutaneous dose, based on U/body surface area), no direct adverse effects on male and female fertility or general reproductive performance was observed."

Based on the internal discussions, sponsor's issues/arguments, and Committee's recommendations, we are proposing the following label in response to sponsors proposed label on 11/5/99.

Carcinogenicity, Mutagenicity, Impairment of Fertility

Standard 2-year carcinogenicity studies in animals have not been performed to evaluate the carcinogenic potential of NovoLog™. In 52 week studies with rats dosed subcutaneously with NovoLog™

The incidence of mammary tumors for NovoLog™ was not significantly different than regular human insulin. The relevance of these findings to humans is not known.

NovoLog™ was not genotoxic in the following tests: Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, in vivo micronucleus test in mice, and in ex vivo UDS test in rat liver hepatocytes. In fertility studies in male and female rats, at subcutaneous doses up to 200 U/kg/day (approximately 32 times the human subcutaneous dose, based on U/body surface area), no direct adverse effects on male and female fertility, or general reproductive performance of animals was observed.

Pregnancy: Teratogenic effects: Pregnancy category C

Subcutaneous reproduction and teratology studies have been performed with NovoLog™ and regular human insulin in rats and rabbits. In these studies, NovoLog™ was given to female rats before mating, during mating, and throughout pregnancy, and to rabbits during organogenesis. The effects of NovoLog™ did not generally differ from those observed with subcutaneous regular human insulin. NovoLog™, like human insulin, caused pre- and post-implantation losses and visceral/skeletal abnormalities in rats at a dose of 200 U/kg/day (approximately 32 times the human subcutaneous dose, based on U/body surface area) and in rabbits at a dose of 10 U/kg/day (approximately three times the human subcutaneous dose, based on U/body surface area). The effects are probably secondary to maternal hypoglycemia at high doses. No significant effects were observed in rats at a dose of 50 U/kg/day and rabbits at a dose of 3 U/kg/day.

These doses are approximately 8 times the human subcutaneous dose for rats and equal to the human subcutaneous dose for rabbits, based on U/body surface area.

NovoLog™ should be used — pregnancy only if the potential benefit justifies the potential risk to the fetus

Nursing mothers- It is unknown whether _____ is excreted _____ in human milk.

Justification for the changes are as follows:

1. **Carcinogenicity:** The statistician's review confirmed that the mammary gland tumor findings with X14 (or regular human insulin) were significantly greater than controls, but only at a high dose (both at 200 U/kg/day)
2. The mammary gland tumor findings with X14 were slightly, but not statistically greater than insulin ($p=0.062$). However, we believe this should be in the label to describe the findings, at 32-times the human dose (from a QA certified study).
3. If the sponsor would like to have the mammary tumor findings removed from the label, they could conduct an adequate 2-year carcinogenicity study and demonstrate that the tumor findings are not significant.
4. **Pregnancy:** Sponsor is right, findings in this category with NovoLog and insulin at similar doses are similar, and this is indicated in the label. Lys-pro (or humalog) has category B labeling in pregnancy, since it was only tested at maximum doses of 20 U/kg/day (which is equivalent to the lowest dose of NovoLog), and findings at these doses may be comparable. Novolog was tested at doses up to 200 U/kg/day in rats, and up to 10 U/kg/day in rabbits, and these doses caused pre- and post-implantation losses and visceral/skeletal abnormalities in rats and rabbits. Therefore, technically as indicated earlier, according to CFR, this makes it a pregnancy category 'C'.
Note that Repro Tox Committee also recommended a "C".

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5. Currently the teratogenic mechanisms that produce these effects in diabetic pregnancies with insulin/analogues are not understood. It is possible that maternal hypoglycemia and /or, more subtle changes in carbohydrate metabolism and/or genetic factors may be important during organogenesis.

/S/

Indra Antonipillai
Pharmacologist, HFD-510

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cc: NDA Arch
HFD510
HFD510/antonipillai/steigerwalt/koller/jrhee
Filename: _____

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**CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT
AND
FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET**

P/T REVIEWER(s):	Indra Antonipillai
DATE:	December 14, 1999
NDA:	20-986
DIVISION(s):	Division of Metabolic and Endocrine drug Products (HFD-510)
DRUG NAME(s):	Insulin aspart (insulin X14, NovoLog)
SPONSOR:	Novo Nordisk Pharmaceuticals Inc.
LABORATORY:	Rat study: _____

TOXICITY STUDY REPORT DATES: November 16, 1994 (QA Rat Study 940301).
Also another Non-QA Rat Study (#930803) was conducted.

THERAPEUTIC CATEGORY: Treatment of diabetes

PHARMACOLOGICAL CLASSIFICATION: Insulin analog
GENOTOXICITY/CLASTOGENICITY: Negative assays:
Ames, mouse lymphoma cell forward gene mutation, human peripheral blood

lymphocyte chromosome aberration, in vivo micronucleus in mice, and in ex vivo UDS
in rat liver hepatocytes

The standard 2-year bioassay to determine the carcinogenicity of the drug (X14) in rats and/or mice have not been performed. However, the incidence of mammary gland tumors was examined in two 1-year toxicity studies in rats in this NDA, with a high dose insulin comparator arm. This is because X14 (and insulin) can act as a growth factor, and like insulin, it has a growth promoting potential. One of these studies was an exploratory study in female rats only (draft and not the final QA report was provided for this study).

STUDY A: RAT CARCINOGENICITY STUDY (Non-QA rat study; Study 930803):

RAT STUDY DURATION (weeks):	52 weeks
STUDY STARTING DATE:	—
STUDY ENDING DATE:	—

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RAT STRAIN:

ROUTE:

DOSING COMMENTS:

NUMBER OF RATS:

Female Sprague-Dawley Crl:CDBR

Subcutaneous injections

Once daily

	Control- (C):	20
NovoLog -	High Dose (NHD):	20
Human insulin-	High Dose (IHD):	20
	High Dose (XHD):	20

RAT DOSE LEVELS (mg/kg/day):

NovoLog- High Dose: 200 U/kg/day, once a day

Human Insulin-High Dose: 200 U/kg/day, once a day

— High Dose: 200 U/kg/day, once a day

BASIS FOR DOSES SELECTED: Not provided, because it was a toxicity study

PRIOR FDA DOSE CONCURRENCE: N/A

RAT CARCINOGENICITY: Positive (females)

RAT TUMOR FINDINGS (details): Females:

Mammary tumors (benign): Incidence

(Con-NHD-IHD-XHD): 4/20 (con)-7/18 (NovoLog)-8/17* (human insulin)-11/17** —

Trend analysis: *P = <0.05 for human insulin. **p=<0.01 for — No significant differences were observed with NovoLog vs controls p=0.16, or between NovovoLog and human insulin at 200 U/kg/day.

Mammary tumors (adenocarcinoma with no
metastasis): Incidence

(Con-NHD-IHD-XHD): 1/20-4/18-3/17-3/17.

Thus, in study A, — at 32 times the maximum human dose, based on body surface area) was associated with higher incidence of mammary tumors than vehicle control. However, this study indicated that the tumorigenic potential of X14 was no greater than endogenous insulin (both at 32 times the maximum human dose, based on body surface area).

STUDY B: RAT CARCINOGENICITY STUDY (QA rat study: Study 940301):

RAT STUDY DURATION (weeks):

52 weeks

STUDY STARTING DATE:

11/16/94

STUDY ENDING DATE:

—

RAT STRAIN:

Sprague-Dawley Crl:CDBR

ROUTE:

Subcutaneous injections

DOSING COMMENTS: Twice daily, 4 hrs apart
 NUMBER OF RATS:
 NovoLog- Control- (C): 32
 - Low Dose (LD): 32
 - Middle Dose (MD): 32
 - High Dose (HD): 32
 Human Insulin-High Dose (HDI) 32

RAT DOSE LEVELS (mg/kg/day):

NovoLog Low Dose: 5 U /kg/bid (or 10 U/kg/day)
 Middle Dose: 25 U /kg/bid (or 50 U/kg/day)
 High Dose: 100 U /kg/bid (or 200 U/kg/day)
 Human Insulin-High Dose : 100 U /kg/bid (or 200 U/kg/day)

BASIS FOR DOSES SELECTED: Not provided, because it was a toxicity study
 PRIOR FDA DOSE CONCURRENCE: N/A

RAT CARCINOGENICITY: Positive (females)

RAT TUMOR FINDINGS (details): Females:

Mammary tumors (benign +malignant): Incidence
 (Con-LD-MD-HD-HDI): 7/32-11/32-11/32-11/32*-6/32^a, Trend analysis: *P = 0.003
 (statistically significant, but only at high dose), ^a p=0.062-Two tailed comparisons of
 NovoLog against human insulin both at 200 U/kg/day.

RAT STUDY COMMENTS: Due to excess mortality at 100 U/kg/bid
 (or total dose of 200 U/kg/day) in groups 4 and 5, in week 25 the daily dose was
 reduced to 100 U/kg/day, and all groups were changed from twice to once daily dose.
 In week 38, in groups 4 & 5, the drug was further reduced to 75 U/kg/day .

RAT STUDY COMMENTS: Note that above two 1-year studies non-QA (study A) and
 QA (study B) were not identical, and there were following differences in these two
 studies: 1) The doses were once a day in study A (200 mg/kg/day), and twice a day in
 study B (100 mg/kg/bid, total dose 200 mg/kg/day). 2) The final X14 doses were
 different in study A (200 mg/kg/day) vs study B (75 mg/kg/day). 3) At termination (1-
 year), the animal survival in study A was 85% (even though these animals were
 receiving 200 U/kg/day throughout the study) vs 44% in study B (even when the doses
 were reduced from 200 to 100 U/kg/day in week 25, and then to 75 U/kg/day in week
 38), no explanation was provided. 4) Study A was a non-QA draft report (we do not
 know the validity of the data), whereas study B was a QA report. 4) Both 1-year studies
 were conducted in mammary prone Sprague-Dawley CD rats. Therefore, these

discrepancies may explain the final differences or outcome, in the above two 1-year studies in rats.

Question to CAC: should the mammary gland tumor findings be listed in the label (see proposed label on page 13).

Study A (non-QA study) : In the first exploratory 1-year toxicity study in rats the effects of _____ were also examined with X14 and human insulin (actrapid). Unlike X14, the _____ has higher affinity for the insulin receptor (205%), compared to both human insulin (100%) or X14 (92%). _____ also has higher affinity for the IGF-1 receptor (0.2%), compared to both, human insulin (0.03%) or X14 (0.05%, affinity of IGF-1 to IGF-1 receptor was 100%), thus affinity of _____ for the IGF-1 receptor is 4-fold higher than that of X14. In this study A (n=20/group, all doses of the drug were given at 200 U/kg/day), the number of rats that had benign tumors were 4/20 (controls), 7/18 (X14), 11/17 _____ and 8/17 (actrapid). The malignant tumors in these groups were 1/20, 4/18, 3/17 and 3/17 resp. Combined analysis (by Peto et al) of total benign + fatal adenomas indicated that _____ was associated with higher incidence of tumors (p<0.01), actrapid was also positive compared to control (p<0.05). The total number of benign mammary tumors (multiple tumors per rat) were 6/20 (controls), 11/18 (x14), 26/17 _____ and 11/17 (actrapid). Thus, in study A, _____ at 32 times the maximum human dose, based on body surface area) was associated with higher incidence of mammary tumors than vehicle control, but this study indicated that the tumorigenic potential of X14 was no greater than endogenous insulin (both at 32 times the maximum human dose, based on body surface area).

Study B (QA study):

One Year-Toxicity Study in Rats after Twice Daily Subcutaneous Injection, (Study No. 940301):

Sponsor's ID Study #: 940301

Amendment #, Vol #, and page # : Volume 19, page 1, Tk-volume 28, page 233

Conducting laboratory : _____

_____ Sponsor: Novo Nordisk A/S, Denmark.

Date of study initiation: November 16, 1994

GLP compliance: Yes

QA Report: Yes (X) No (), Is the evaluation based on a final QA report: Yes

Methods: This study compared the effects of the new drug X14 (at 5, 25, and 100 U/kg/bid) vs with recombinant human insulin HM(ge) at 100 U/kg/bid in rats, for 52 weeks.

Dosing information:

species: Crl:CD BR rats

#/sex/group or time point: 32/sex/group

age: ~26-28 days old

weight: Males 79-91 g, females 72-85 g.

satellite groups used for toxicokinetics: 9/sex/group were used for TK

Dosage groups in administered units: Four groups (20 rats/sex/group) were given either vehicle (0.15% phenol, 0.172% M-cresol and 1.60 % glycerol _____), or X14 (an old process drug, i.e process A) twice daily by subcutaneous injections at doses of 5, 25, 100 U/kg/bid (or total dose of 10, 50, 200 U/kg/day) for 52 weeks. The fifth group received HM(ge) at twice daily dose of 100 U/kg/bid (or total dose of 200 U/kg/day). Additional 5 satellite groups (n=12/sex/group) received the drug similarly for TK studies, but since the animals were dying at 200 U/kg/day in group 4 and 5, satellite animals were also included in the main study and their doses were reduced. In week 25 (day 1), in groups 4 and 5, the drug was reduced by 50% to 50 U/kg/bid. In week 27 (day 4), all groups changed from twice to once daily dose, and for groups 4 and 5, the daily dose was 100 U/kg/day. In week 38 (day 1), in groups 4 and 5, the drug was further reduced to 75 U/kg/day (see appendix). Full necropsy and histology were performed on TK animals as well.

Route, form, volume, and infusion rate (if i.v.): Subcutaneous injections were given twice daily, 4 hrs apart, at a volume of 0.5 ml/kg, for up to weeks 25, and after that once daily for up to week 52.

Drug, lot #: X14 old process drug, Batch No. 06594 _____, 06894 _____, and 07094 _____ HM(ge) Batch # 06194 _____

Formulation/vehicle: Vehicle was 0.15% phenol, 0.172% M-cresol and 1.60 % glycerol _____

Times at which Observations are made:

Clinical signs: Daily

Body weights: At The time of allocation to groups, on the day of Tx and once a week thereafter.

Food consumption: Weekly.

Ophthalmoscopy: Before treatment and during weeks 13, 26, and 52 of treatment.

Hematology: Prior to the first dose and at the start of week 4

Clinical chemistry: Prior to the first dose of the morning during weeks 12, 25, and 51 of treatment

Urinalysis: Overnight urine samples were collected during week 12 and 25.

Gross pathology: At sacrifice at 52 weeks.

Organs weighed: These are highlighted in the histopathology table

Histopathology:* At sacrifice (from controls and HD and animals that died). Incidences of mammary tumors were analyzed. Testes and epididymides were stained with

Periodic Acid Schiff (PAS) reagent. Liver sections were stained with Oil-O-Red O, and with PAS for glycogen. Also mammary gland (MG), lymph nodes from MG, and subcutaneous masses were examined in all animals.

Toxicokinetics (in satellite animals): Days 1 and after weeks 13, 26 and 52 of treatment, at 0, 1, 3, 4, 5, and 8 hr. Plasma glucose levels were also measured in these animals.

Other (antibody determination in main study animals): Prior to first dose, and during weeks 12, 25 and 51.

Results:

Mortality: In all groups there were 32 rats/sex/dose, including 12 rats from satellite group. 18 of 32 male, and 18 of 32 female animals died in the high dose groups, thus mortality was 56% at a high dose (Table 8). In the mid dose group 25% of female rats died, while only 12% of male rats died as shown below. Sponsor indicates that total drug related deaths were 0, 1, 3, 17 and 20 in males, and 0, 1, 6, 15 and 15 in females at 0, 10, 50 and 200 U/kg/day of X14, and 200 U/kg/day of HM(ge) resp. Thus there were similar number of deaths with the drug X14, or with regular human insulin, at high dose. The animals were found dead prior to dosing at the morning check. Pathological findings showed hypoglycemia at the time of death or empty GI contents and haemorrhagic depression/erosions of the stomach wall.

Table 8. Mortality data in 1-year rat toxicity study:

Week	X14 Dosage(U/kg/day) in Male				X14 Dosage(U/kg/day) in Female			
	Control	10	50	200	Control	10	50	200
N	32	32	32	32	32	32	32	32
1-24	0	0	2	8	2	1	3	11
25-27	0	0	0	2	1	0	0	1
28-37	1	0	0	4	0	0	0	1
38-53	1	2	2	4	5	0	5	5
1-54	2	2	4	18	3	6	8	18
# Surv.	30	30	28	14	29	26	24	14
% Surv.	94	94	88	44	91	81	75	44

#Surv and % surv = Indicate the number and % of animals survived at the end of 52-week study. Due to the number of deaths in the high dose group, the dosing regime was amended as follows: Day 1 Week 25, the high dose reduced by 50%; Day 1 Week 38, the high dose reduced by 25% to 75 U/kg/day.

Clinical Signs: A total of 19 male and 23 female rats experienced apparent hypoglycemic episodes (between 2-4 hrs after dosing). Plasma glucose in these rats was < than 2 mmol/l, and administration of glucose did not prevent the subsequent

death in most of these rats. Following changes in dose regimens in weeks 25, 27 or 38 did not improve the incidence of hypoglycemic episodes.

Body weight and Food Consumption: During the first 24 weeks of the study, there was an increase in body weight gain for rats of either sex receiving 50 and 200 U/kg/day. After the 50% reduction in dosage in the high dose group after 24 weeks of treatment, a transient weight loss was seen (up to 30%). However, the final reduction in X14 dose after 37 weeks of treatment showed no clear change in growth pattern, which indicates that there is no obvious relationship between the insulin dose and bodyweight changes. Food consumption for both sexes at the low and the mid dose groups was essentially similar to the controls, although the high dose increased the food consumption by 5-15% in female and 10 to 20% in male rats. Likewise, water consumption increased 10 to 20% in males only.

Ophthalmic Examination: There were no ocular changes considered related to treatment, although the number of survivors in the high dose group was rather small.

Hematology: In weeks 12 and 25 there was a slight (< 10%) reduction in red cell counts in males of the high dose group with the drug or HM(ge), which was not statistically significant. In contrast, MCH and MCHC were increased (by 5-9%) at high doses with both, the drug or HM(ge).

Biochemistry: Triglyceride (TG) increased at 200 U/kg/day, in both sexes in weeks 12, 25 and 51, values are shown at 51 weeks at 0, 10, 50, 200 U/kg/day with X14, and 200 U/kg/day with HM(ge) resp. Samples taken for clinical chemistry showed increased glucose levels in treated animals compared to controls, which is contrary to what is indicated under the mean plasma glucose concentrations below (which were decreased 1- hr after the drug dosing in a separate sampling). No explanation was provided by the sponsor for this discrepancy, except that the timing of blood sampling may have been different, and may explain it.

Parameter	males	females
glucose	124, 144, 176, 170, 185	132, 140, 139, 159, 164
TG	137, 164, 146, 194, 166	125, 106, 97, 147, 141
Urinary volume	5.9, 7.1, 8.7, 9.9, 10.5	7.1, 6.1, 8.7, 9.7, 6.6

Urine analysis showed increased urinary volume, along with decreases in total protein, and there was evidence of glycosuria in some animals at high doses of the drug or insulin, which may be due to high blood glucose levels.

Mean plasma glucose concentration: These were decreased in all treated groups to a similar level (4-5 mmol/l), one hour after the first dose on Day 1 and Weeks 13 and 26. Recovery by 4-5 hrs was initially observed, but in mid-high dose groups the plasma glucose remained low at all time points after the first few days. At Week 52, the glucose

level at 1 hour post dose was slightly lower for all treated groups (males:3-4, females:2-4 mmol/l).

Organ Weights: At 200 U/kg/day, absolute weights of following organs were altered:

In males: heart (2.15 vs 1.91 g in controls), prostate (1.1 vs 1.42 g in controls), salivary gland (0.63 vs 0.81 g in controls), seminal vesicle (1.5 vs 1.9 g in controls). The relative heart weights at these high doses were increased by 8% and prostate weights decreased by 25%.

In females: brain (2.01 vs 2.09 g in controls), uterus (1.1 vs 0.69 g in controls), and ovaries (110 vs 90.9 g in controls). The relative uterus and ovary weights at these high doses were increased by 50% and 10% resp.

Gross pathology: An increased incidence of subcutaneous masses in mammary gland region and mammary gland cysts were observed in female rats (In both dead rats, and animals sacrificed at the end of study). Haemorrhagic depressions were observed in the stomach corpus mucosa in both sexes. The n=32/group includes both, the dead animals and those that survived till terminal sacrifice.

In females

Subcutaneous masses	7/32, 12/32, 15/32, 11/32, 7/32
Mammary gland cysts	6/32, 7/32, 5/32, 3/32, 9/32
Haemorrhagic depressions	0/32, 1/32, 4/32, 6/32, 8/32

In males

Haemorrhagic depressions	1/32, 2/32, 1/32, 10/32, 12/32
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Histopathology: The time-to-tumor method (Peto et al, 1980^a) was used to examine the number of animals with mammary tumors (MT). The incidence of animals with benign and malignant mammary gland tumors combined ($p=0.003$) or benign mammary gland tumors alone ($p=0.0039$) were significantly higher at 100 U/kg/bid of X14 in rats compared to controls. Malignant mammary tumors were also increased with 100 U/kg/bid of X14, but it was not significant ($p=0.090$). However, the incidences of these mammary tumors was not significantly increased in animals treated with HM(ge) at 100 U/kg/bid compared to controls (in a similar analysis). **Slight but not significant ($p=0.062$, two tailed comparison with HM(ge)) increases in the incidence of benign + malignant mammary gland tumors was observed with X14 compared to HM(ge) at 100 U/kg/bid.** No differences in total mammary gland tumors were noted between controls and 200 U/kg/day with X14 or HM(ge). Erosion of the glandular epithelium was noted in dead animals at 50 and 200 U/kg/day, these animals had died from hypoglycemia. Also focal seminiferous atrophy, often associated with vacuolation of sertoli cells was noted in the testis of male animals, although some of these were also noted in controls. The testicular changes with the drug may be due to disrupted lactate metabolism in sertoli cells by insulin-induced hypoglycemia, as developing

spermatocytes are dependent on lactate as an energy source. Pituitary adenoma (benign) and/or hyperplasia was observed in all animals but these were not significantly different between control and with 100 U/kg/bid of X14 or HM(ge). Sponsor states that the increase in mammary tumors observed in this study was also noted in 2 previous studies with insulin or — in female rats (one was an exploratory study in female rats, see toxicity summary, the data on the other study were not provided), and that these compounds cause mammary tumors in mammary prone female Sprague-Dawley CD rats. However, this study suggests that the incidence of mammary tumors with X14 may be higher than with human insulin, and further studies may be required to clearly establish its role in the induction of mammary gland tumors

^a = Peto R et al, In "long-term and short term screening assays for carcinogens: a critical appraisal. IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Supplement 2, page 311-426. International Agency for Research on Cancer, Lyon, 1980.

Doses (U/kg/day): 0,10, 50, 200 of X14, and 200 of HM(ge)

Males

Stomach (erosion of stomach in dead rats)

Erosion of glandular epithelium 0/2, 1/1, 2/4, 8/18, 12/20

Testes (seminiferous tubular changes)

Focal seminiferous tubular epithelial atrophy 0/32, 0/1, 0/4, 4/32, 3/32

Vacuolation of sertoli cells 0/32, 0/1, 0/4, 2/32, 2/32

seminiferous tubular atrophy 4/32, 0/32, 0/4, 1/32, 1/32

Pituitary

Adenoma (benign) and/or hyperplasia 6/32, 0/1^b, 2/4^b, 7/32, 9/32

Females

Mammary tissue (n= 32/dose)

1. Rats bearing mammary tumors (benign+ malignant) 7, 11, 11, 11*, 6^c

2. Rats with benign tumors (fibroadenoma/adenomas) 6, 9, 10, 8**, 5

3. Rats with malignant tumors (adenocarcinomas) 2, 4, 2, 4***, 1

4. Rats with more than one MT 4, 2, 2, 3, 3

5. Total number of Mammary tumors 11, 13, 13, 14, 15

Stomach (erosion of stomach in dead rats)

Erosion of glandular epithelium 0/3, 0/4, 1/3, 4/18, 5/17

Pituitary

Adenoma (benign) and/or hyperplasia 6/32, 1/4^b, 0/3^b, 7/31, 8/32

* p= 0.003 compared to controls (using the trend test for all mammary tumors).

**p=0.0039 compared to controls (using the trend test for benign mammary tumors)

^{***}p= 0.090 compared to controls (using the trend test for malignant mammary tumors)

^b= At low-mid doses these were examined only in dead animals.

^cp=0.062- two tailed comparisons of X14 (200 U/kg/day) against HM(ge) at 200 U/kg/day.

Antibody Determination (study # 950183): Antibodies against X14 or HM(ge) were found in majority of all treated animals in both sexes in week 12 (7/10, 10/10, 9/10, 8/10 at 10, 50, 200 and 200 U/kg/day resp), but decreased in week 25 and 51 (1/10, 5/10, 5/10, 5/10). Antibody presence did not neutralize the effects of X14 or HM(ge), as no obvious effects on glucose were observed, i.e. hypoglycemic response was maintained throughout the study.

Toxicokinetics (study # 960270). Highest insulin X14 levels were noted at 1 hr (M+F nM at 10, 50, and 200 U/kg/day resp) and 5 hrs. No gender differences were noted and linearity in Cmax and doses were observed. The rate of elimination of the drug did not increase with time, however no tabular data were provided, only figures were given (see appendix).

Conclusions: Treatment of the rats with the old X14 (at doses of 0, 5, 25, 100 U/kg/bid) and human insulin (HM(ge) 100 U/kg/bid) for 1-year caused dose related mortality in animals (deaths were 0, 2, 9, 32, and 35 in five above groups resp due to hypoglycemia, associated with empty GI contents and hemorrhagic depression/erosions in the stomach wall, which were noted in 0, 1, 3, 7, and 17 dead animals resp). Since more animals were dying at 200 U/kg/day in group 4 and 5, in week 25 in groups 4 and 5 the drug was reduced by 50% to 50 U/kg/bid. In week 27, all groups were changed from twice to once daily dose, and for groups 4 and 5, the daily dose was 100 U/kg/day. In week 38, in groups 4 and 5, the drug was further reduced to 75 U/kg/day. In both sexes the drug caused, clinical signs (hypoglycemic episodes), increase in food and water consumptions (by 10-20%), and biochemical changes (TG increased by 18-42% and 13-21% with X14 and HM(ge) at 100 U/kg/bid, plasma glucose conc decreased with all drug doses at 1-hr after dosing. Plasma glucose in the mid-high dose groups stayed low at all times, after first few days of dosing). The drug caused increased incidence of subcutaneous masses in mammary gland region (7, 12, 15, 11, & 7 at 0, 10, 50, 100 of X14 U/kg/bid or HM(ge) resp). Haemorrhagic depressions were observed in the stomach corpus mucosa in both sexes (1, 3, 5, 16 & 20 resp). Histopathology showed that the incidence of animals with benign + malignant mammary gland tumors (7, 11, 11, 11*, 6 at 0, 10, 50, 100 of X14 U/kg/bid or HM(ge) resp), or benign mammary gland tumors alone (6, 9, 10, 8*, and 5 resp) were significantly higher at 100 U/kg/bid of X14, compared to vehicle controls. These were not significantly increased at similar doses of HM(ge), compared to controls, and no significant differences in the incidence of mammary tumors was observed between X14 and HM(ge) at 100 U/kg/bid. The number of malignant tumors were higher with X14 at 100 U/kg/bid (4 vs 2 in vehicle controls, p=0.09), but not significantly increased. However no differences in total number of mammary gland tumors were noted between controls vs 200 U/kg/day of X14

or HM(ge), i.e. 14 and 15 resp vs 11 in controls. Sponsor states that the increase in mammary tumors with X14 seen here was also noted in 2 previous studies with insulin or — in female CD rats (NN study # X14/930308, and NN study # actrapid/940267), this suggests that these compounds cause mammary tumors in mammary prone female Sprague-Dawley CD rats (i.e. lobular hyperplasia with an increase in the number of acini in a mammary lobule in this strain, at this age). Also seminiferous tubular (ST) atrophy in testes of male animals was observed in both treated and controls, however focal ST epithelial atrophy, associated with vacuolation of sertoli cells was only noted in the drug (or with human insulin) treated rats. The testicular changes with the drug may be due to disrupted lactate metabolism in sertoli cells by insulin-induced hypoglycemia, as developing spermatocytes are dependent on lactate as an energy source. Antibodies against X14 or HM(ge) were found in majority of treated animals in both sexes in week 12 (7/10, 10/10, 9/10, 8/10 at 10, 50, 200 and 200 U/kg/day resp), but decreased in week 25 and 51 (1/10, 5/10, 5/10, 5/10). Antibodies had no effects on the reduction of glucose levels or on hypoglycemic related deaths, suggesting that antibodies did not neutralize the effects of X14 or human insulin. The 'no toxic effect dose' could not be determined in this study, as deaths also occurred at lower doses of drug, due to insulin-induced hypoglycemia. The effects of X14 and human insulin in general were similar in this 1-year toxicity study in rats.

In summary in study 'B' (GLP and QA certified study), in 1-year toxicity study in rats at 100 U/kg/bid of X14 (or total dose of 200 U/kg/day, 32 times the maximum human dose, based on body surface area), the incidence of benign mammary gland tumors alone ((8/32* vs 6/32 in controls), or benign with malignant tumors combined (11/32* vs 7/32), was significantly higher with X14 compared to vehicle controls (*p=0.003). The number of malignant tumors alone, was also higher with X14 at 100 U/kg/bid (4 vs 2 in vehicle controls, but not significantly increased compared to controls, p=0.090, with human insulin these were lower than controls, i.e. 1 vs 2 in vehicle controls). Slight but not significant (p=0.062) increases in the incidence of benign + malignant mammary gland tumors were observed with X14 compared to regular human insulin, both given at 100 U/kg/bid.

Note that the above two 1-year studies A and B were not identical, as indicated on page 10

No carcinogenicity studies with X14 have been conducted, sponsor states that like insulin, this drug is a large protein, and insulin has been in clinical use for 50 years, with no epidemiological link with cancer in man. Also they state that Sprague Dawley CD rats are prone to spontaneously developing mammary gland neoplasms at 1 year of age (note that humans are also mammary prone species), and given the growth promoting effects of the drug (or insulin), it is not surprising that the marginal increase in these tumors is noted, but no other neoplastic lesions were found in the regular 1-year rat/dog studies. Studies on the mutagenic potential of X14 and regular human insulin do not

NDA 20-986

show any significant differences between the two products. The affinity of X14 for the insulin receptor is similar to that of human insulin (92% with X14, vs 100% with human insulin), and its affinity for the IGF-1 receptor is slightly higher but not significantly different from human insulin (0.05% with X14, 0.03% with human insulin, 0.2% with vs 100 % with IGF).

**APPEARS THIS WAY
ON ORIGINAL**

Executive CAC

December 21, 1999

Committee: Paul Andrews, Ph.D., HFD-150, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Bob Osterberg, Ph.D., HFD-520, Alternate Member
Ron Steigerwalt, Ph.D., Team Leader
Indra Antonipillai, Ph.D., HFD-510, Presenting Reviewer
Adele Seifried, Project Manager

Author of Draft: Indra Antonipillai, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA 20-986
NovoLog (X14, insulin aspart)
Novo Nordisk Pharmaceuticals Inc.

Rat carcinogenicity study.

NovoLog is an insulin analog for treatment of diabetes. NovoLog has a single amino acid substitution compared to human insulin (aspartic acid for proline). Its pharmacological activity is supposedly similar to insulin, but with a faster onset of action.

No carcinogenicity studies with NovoLog (X14) have been conducted. The sponsor states that insulin is a growth factor, and like insulin, this drug is a large protein. Since insulin has been in clinical use for 50 years, with no epidemiological link with cancer in man, the 2-year standard carcinogenicity studies with NovoLog were not required. The sponsor, however, has conducted two 1-year toxicity studies with NovoLog in rats (one draft study A, and the other QA study B), and found mammary tumors.

In a 1-year draft toxicity study A, NovoLog, human Insulin, and _____ were given to rats for 1-year at 200 U/kg/day, once a day. Female rats had mammary tumors (benign), with incidence: 4/20 (con), 7/18 (NovoLog), 8/17* (human insulin), 11/17** —, *P<0.05 for human insulin. **P<0.01 for — No significant differences were observed with NovoLog versus controls P=0.16, or between NovoLog and human insulin at 200 U/kg/day in this study. Another

_____ was associated with a higher incidence of mammary tumors than the vehicle control. This study indicated that the tumorigenic potential of X14 was no greater than endogenous insulin (both at 32 times the maximum human dose, based on body surface area). _____

In a 1-year QA toxicity study B, NovoLog, (10, 50 and 200 U/kg/day), was given along with human insulin (as comparator at a high dose of 200 U/kg/day) to rats for 1-year, twice a day. The incidence of female rats with mammary tumors (benign+malignant) was: 7/32 (con), 11/32 (low dose NovoLog), 11/32 (mid dose NovoLog), 11/32* (high dose NovoLog), 6/32 (high dose insulin); *P=0.003 for trend analysis (statistically significant compared to vehicle control, but only at high dose) and P=0.004 for one-tailed pair-wise comparison to control, **P=0.062 for two tailed comparisons of NovoLog against human insulin both at 200 U/kg/day (no statistically significant difference). Our statistician's review confirmed that the mammary gland tumor findings with X14 were significantly greater than controls, at the high dose of NovoLog (200 U/kg/day). The incidence of benign + malignant mammary tumors was 7, 11, 11, 11* with NovoLog, and 6 with regular human insulin. The P values at the high dose were 0.003 for the trend test and 0.004 for the pair-wise comparison to control. The incidence of benign tumors were also significantly increased at a high dose of NovoLog 6, 9, 10, 8* and 5 in the above five groups respectively (The P values at the high dose were 0.039 for the trend test and 0.051 for the pair-wise comparison to control). Neither value was below the level of significance for a common tumor in a 2 year bioassay. In this study, NovoLog at 200 U/kg/day was associated with a higher incidence of mammary tumors than vehicle control. Nonetheless, no significant differences in mammary tumors were observed between NovoLog and regular human insulin (P=0.062, both at 32 times the maximum human dose, based on body surface area). Note that due to excess mortality at 100 U/kg/bid (or total dose of 200 U/kg/day) in groups 4 and 5, in week 25 the daily dose was reduced to 100 U/kg/day, and all groups were changed from twice to once daily dose. In week 38, in groups 4 & 5, the drug was further reduced to 75 U/kg/day.**

RAT STUDY COMMENTS: Note that both 1-year studies were conducted in mammary tumor prone Sprague-Dawley CD rats and that studies A (non-QA) and B (QA'd) were not identical. The following differences in these two studies may explain the final differences in some study outcomes:

1. The doses were once a day in study A (200 U/kg/day), and initially twice a day in study B (100 U/kg/bid, total dose 200 mg/kg/day).
2. The final X14 doses were different in study A (200 U/kg/day) versus study B (75 U/kg/day).
3. At termination (1-year), the animal survival in study A was 85% (even though these animals were receiving 200 U/kg/day throughout the study) versus 44% in study B

(even when the doses were reduced from 200 to 100 U/kg/day in week 25, and then to 75 U/kg/day in week 38), no explanation was provided.

4. Study A was a non-QA draft report (we do not know the validity of the data), whereas study B was a QA report.
5. The numbers of rats/sex/group were different (32 versus 20).

Proposed Label Text by the sponsor on 11/5/99 amendment:

"Carcinogenicity, Mutagenicity and Impairment of Fertility

In 52-week studies with Sprague-Dawley rats dosed subcutaneously with NovoLogTM NovoLogTM was not genotoxic in the following 5 different tests; Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, in vivo micronucleus test in mice, and in ex vivo UDS test in rat liver hepatocytes. In fertility studies in male and female rats at subcutaneous doses up to 200 U/kg/day (approximately 32 times the human subcutaneous dose, based on U/body surface area), no direct adverse effects on male and female fertility or general reproductive performance was observed."

Executive CAC Recommendations and Conclusions:

2. The Committee concurred that although the mammary gland tumor findings with X14 and insulin were not significantly different, that they were significantly greater than the vehicle control in study B. The Committee recommended that the positive findings with both X14 and insulin be reported in the label with a statement that the clinical relevance of these findings is unknown.

3. The Committee concurred with the following new proposed labeling for NovoLog.

Carcinogenicity, Mutagenicity, Impairment of Fertility

Standard 2-year carcinogenicity studies in animals have not been performed to evaluate the carcinogenic potential of NovoLogTM. In 52 week studies with rats dosed subcutaneously with NovoLogTM

The incidence of mammary tumors for NovoLogTM was not significantly different than regular human insulin¹⁰². The relevance of these findings to humans is not known. NovoLogTM was not genotoxic¹⁰⁴ in the following tests: Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte

chromosome aberration test, in vivo micronucleus test in mice, and in ex vivo UDS test in rat liver hepatocytes. In fertility studies in male and female rats, at subcutaneous doses up to 200 U/kg/day (approximately 32 times the human subcutaneous dose, based on U/body surface area), no direct adverse effects on male and female fertility, or general reproductive performance of animals was observed¹⁰⁵.

4. {

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Paul Andrews, Ph.D.

Acting Chair, Executive CAC

cc:/

/Division File HFD-510, NDA 20-986
/HFD-510/Antonipillai/Steigerwalt/Rhee
/ASeifried, HFD-024

**APPEARS THIS WAY
ON ORIGINAL**

Reproductive Toxicity Assessment Committee (RTC) Report

Cover Sheet

Review of Reproductive Toxicity Studies

IND/NDA No.: NDA 20-986
Drug Name: Insulin aspart (insulin X14, NovoLog)

CAS No.: N/A
Division Name/HFD No.: HFD-510
Reviewer Name/Phone No.: Indra Antonipillai, 301-827-6393
Sponsor: Novo Nordisk Pharmaceuticals Inc. _____

Clinical Indications: Treatment of diabetes
Drug Classification: Insulin analog

Date Submitted to RTC: December 20, 1999
Date of RTC Review:
Reviewing RTC Members:

Clearly state the basic question(s) you would like the RTC to answer.:

Should NovoLog pregnancy category label be — 'C' ?

1. Both NovoLog™, and regular human insulin, caused pre- and post-implantation losses and visceral/skeletal abnormalities in rats at a dose of 200 U/kg/day (approximately 32 times the human subcutaneous dose, based on U/body surface area) and in rabbits at a dose of 10 U/kg/day (approximately three times the human subcutaneous dose, based on U/body surface area).
2. Regular human Insulin has no pregnancy category labeling.
3. Another insulin analog (Lispro, approved Eli Lilly drug) has pregnancy category label 'B'. However, it was only tested at maximum doses of 20 U/kg/day in rats and 0.75 U/kg/day in rabbits (which are generally equivalent to the lowest doses of NovoLog), and findings at these doses are generally comparable between Novolog and humalog. However, technically according to CFR, this makes it a pregnancy category 'C'.

This is the proposed labeling by sponsor on NovoLog (amendment 11/8/99)

Pregnancy: Teratogenic effects: Pregnancy category —

Subcutaneous reproduction and teratology studies have been performed with NovoLogTM and regular human insulin in rats and rabbits. In these studies, NovoLogTM was given to female rats before mating, during mating, and throughout pregnancy, and to rabbits during organogenesis. The effects of NovoLogTM did not — differ from those observed with subcutaneous regular human insulin. NovoLogTM, like human insulin, caused pre- and post-implantation losses and visceral/skeletal abnormalities in rats at a dose of 200 U/kg/day (approximately 32 times the human subcutaneous dose, based on U/body surface area) and in rabbits at a dose of 10 U/kg/day (approximately three times the human subcutaneous dose, based on U/body surface area). The effects are probably secondary to maternal hypoglycemia at high doses. No significant effects were observed in rats at a dose of 50 U/kg/day and rabbits at a dose of 3 U/kg/day. These doses are approximately 8 times the human subcutaneous dose for rats and equal to the human subcutaneous dose for rabbits, based on U/body surface area.

Following issues are to be considered for the NovoLog labeling:

1. We had suggested pregnancy category 'C' for NovoLog. Because if you have findings, technically CFR indicates that this pregnancy category should be 'C'.
2. Insulin has no pregnancy labeling (it has been in the market for 80 years), but previous studies have shown that insulin given during pregnancy can induce structural changes in the offsprings in various species, this happens at doses as low as 1 U or less (Schardein J.L. in Chemically-induced birth defects, second edition, Marcel Dekker, Inc., New York and Basel). Furthermore, in pregnant rats, brief hypoglycemia with insulin treatment during organogenesis, can disrupt normal embryo development (J. Clin. Invest. 78: 643, 1986).
- 3 Humalog (lispro) is labeled as pregnancy category 'B'. It is an Eli Lilly drug, which was recently approved, and is another insulin analog. Humalog was tested up to 4 and 0.3 times the human dose in rat and rabbit repro-toxicity studies, and these were the 'no toxic effects doses'. The Humalog doses in rat segment II studies were up to 20 U/kg/day, but no effect dose was 5 U/kg/day (or 4 times the human dose). The review (completed on 2/28/1996)

states that at high dose (20 u/kg/day) marginal effects on utero growth, decreased fetal body weight, and increased incidence of fetal runts/litter were observed (no details on these were listed).

4. Like Humalog, 'no toxic effects doses' of NovoLog in rats and rabbit repro-tox studies were 8 and 0.3 times the human dose. However the sponsor here tested NovoLog up to 32 and 3 times the human doses in rats and rabbits respectively, and these doses produced repro-findings (see attached review).
5. Sponsor states that in the Humalog labeling, the maximum dose of Humalog used was 20 U/kg/day in rats and 0.75 U/kg/day in rabbits, these are 1/10th of NovoLog doses, and their effects are comparable to the lower doses of NovoLog. The sponsor further states that the effects seen with insulin and NovoLog are class-effects, secondary to hypoglycemia, therefore pregnancy classification (category — should reflect findings of the 2 products at similar dose levels, unless clear differences between insulin preparations have been demonstrated.
6. We considered whether hypoglycemia played a role in maternal toxicity. In both rats and rabbit repro-studies, plasma glucose values were decreased with all doses at 1-hr after dosing, but it took more than 4-hrs to recover at high doses (200 U/kg/day in rats, and at 10 U/kg/day in rabbits). This suggested that persistent hypoglycemia may play a role in maternal toxicity. Currently the teratogenic mechanisms that produce these effects in diabetic pregnancies with insulin/analogues are not understood. It is possible that maternal hypoglycemia and /or, more subtle changes in carbohydrate metabolism and/or genetic factors may be important during organogenesis. Our initial label review indicated this, and stated that _____
 _____ However, the sponsor has changed the labels as follows: 'The effects are probably secondary to maternal hypoglycemia at high doses'.
7. Sponsor is right, findings in this category with NovoLog and insulin at similar doses are similar. Lispro (or humalog) has category B labeling in pregnancy, since it was only tested at maximum doses of 20 U/kg/day (which is equivalent to the lowest dose of NovoLog), and findings at these doses are comparable. However, technically as indicated earlier, according to CFR, this makes it a pregnancy category 'C'. Insulin has no pregnancy category labeling, so it makes it difficult to compare.
8. On the approved Lily drug (Lispro or Humalog), where it was pregnancy category 'B', the fetuses with malformations are being reported in some cases, when given to pregnant women. It is not clear if they are drug related at this time

Summary of Reviewer's Recommendations to the RTC:

We agreed with the sponsor, that it should be labeled as category ~~II~~ and not penalize them for testing higher doses.

However, after consultation with the Repro-Tox. Committee, we decided to change to the original recommended pregnancy label to 'C' as outlined on March 6, 2000 review.

**APPEARS THIS WAY
ON ORIGINAL**

REPRODUCTIVE TOXICOLOGY:

Study title: Segment I/II. Effects of Subcutaneous X14 (Twice Daily) On Male/Female Fertility and Embryo-Fetal Development in Rats (Study # 940303)

Study No: 940303

Site and testing facility: _____

GLP compliance: Yes

QA- Reports Yes (X) No ():

Lot and batch numbers: Old process X14 06794 _____ 06994 _____
07194 _____ HM(ge) 06294 _____

METHODS:

Species/strain: Sprague-Dawley rats (Crl:CD BR VAF/plus strain), males 9-10 week old, 259-298 g, females 5-6 weeks old, 129-160 g.

Doses employed: 0, 5, 25 and 100 U/kg/bid (or total doses 0, 10, 50 and 200 U/kg/day)

Route of Administration: Subcutaneous

Number of animals/sex/dosing group: 24

Study Design: This study examined the effects of X14 on fertility and embryo fetal

development in rats. Also a group was included to receive recombinant human insulin HM(ge) at 100 U/kg/bid for comparative purposes. Four groups of 24 male and

female rats were given the three doses of the drug (at 0, 5, 25, and 100 U/kg/bid), or

HM(ge) subcutaneously, twice daily (4 hrs apart). Males and females were treated for

4 and 2 weeks resp prior to mating, and throughout the mating. Males continued to be

treated till termination of all females (till day 20 of gestation). Females continued treatment till day 0-15 of gestation. Mated females were sacrificed on day 20 of pregnancy. Control animals received the vehicle (0.16% phenol, 0.172% m-cresol and

1.60 % , and 0.19% zinc).

Parameters and endpoints evaluated:

Clinical signs : Daily

Body weights/food consumptions: Prior to dosing, and twice weekly. Weights were recorded for days 0,3, 6, 8, 12, 14, 16, 18 and day 20 of pregnancy.

Water consumption: Daily for males in weeks -1, 1 and 3, females week 2 and 3.

Plasma Glucose: On day 1 of treatment and again in week 4 at 0, 1, 4 hrs.

Mating Performance: Vaginal smears were taken for 7 days prior to mating.

Terminal examination of Females: Females were sacrificed on day 20 of pregnancy. The ovaries and uteri were examined. The number of corpora lutea, number of implantation sites, number of live/dead fetuses, and number of pre- and post- implantation losses, and fetal/placental abnormalities were recorded. All fetuses were weighed, sexed and examined for external abnormalities. One half of fetuses were examined for visceral abnormalities by the Wilson's technique, the other half were examined for skeletal malformations and variations using alizarin staining by the modified Dawson's technique.

Terminal examination of Males: The males were killed and examined externally and internally for abnormalities, and adrenals, brain, testes, epididymides, vas deferens, prostate, seminal vesicles and pituitary were weighed. Serum motility, sperm morphology and sperm count were examined.

Statistical evaluations: Analysis of incidence of fetal malformations and anomalies were performed using a one-tailed permutation test.

RESULTS:

Mortality and Clinical signs: One male rat (in week 8), one female rat (on day 16 of pregnancy) at 100 U/kg/bid dose of X14, and one male at 100 U/kg/bid of HM(ge) died due to hypoglycemia, histopathology showed hemorrhagic depressions/foci in the stomach corpus mucosa in the GI tract. One male which died had hypoglycemic episodes prior to death. No other signs were observed

Body weight: In males, during week 0-4 the body weight gains increased (* $p < 0.05$ -0.001) at 25 and 100 U/kg/bid (these were 97%, 108%, 126% and 128% at 5, 25, 100 U/kg/bid of X14 and HM(ge) resp. Similar increases were also noted during week 0-9 in males (100, 109, 126* and 134*% resp). In females, during week 2-4 the body weight gains increased also at 25 and 100 U/kg/bid (these were 96%, 138%, 158*% and 154*% at 5, 25, 100 U/kg/bid of X14 and HM(ge) resp). Similar increases were also noted during week 2-7 in females (101, 105, 108 and 113% resp). No effects on weight gains were observed at 5 U/kg/bid

Food consumption: In both males and females, food consumptions were increased at mid-high doses (males 96, 102, 112*, and 11*5, females 97, 105, 110*, and 113* at 5, 25, 100 U/kg/bid of X14 and HM(ge) resp). Water consumption was increased in both sexes at mid and high doses during the first week of treatment. (310-326 vs 265-292 in controls).

Plasma Glucose Concentrations: On day 1 (1-hr) values are shown below. These were all decreased with the drug. However it took more than 4 hrs to recover the blood glucose at high doses (100 U/kg/bid) with both X14 or HM(ge).

Males	females
8.6, 5.2, 5.0, 4.8, 4.8	8.5, 4.2, 5.3, 5.2, 4.3

Mating Performance: The mating performance was not affected, there were no effects on male or female fertility index or copulation index. Pre-coital time

(during days 6-10) was slightly higher at mid-high doses (1, 1, 4, and 3 at 0, 5, 25 and 100 U/kg/bid resp), while cell type (sperm, cornified epithelial/epithelial leukocyte) at conception was not significantly altered. Most females conceived within five days of pairing.

FERTILITY IN MALES: Pituitary organ weights in males were significantly decreased ($p < .05-0.01$) in a dose related manner (15.2, 14.9, 13.8*, 13.1*, 14.0 in five groups resp). At 100 U/kg/bid, epididymides weight was slightly lower (— vs — in controls), this was also noted with HM(ge) at this dose. Seminology: This showed a decrease in sperm motility at 100 U/kg/bid (95% of controls). sperm count was lower with HM(ge) (184 vs 202 in controls) but not with the drug — vs 202 in controls). However, sperm morphology was not significantly different at 100 U/kg/bid with the drug or HM(ge) (93.6 vs 96.6 with controls). Gross pathology in males showed higher incidence of pale subcapsular areas in the liver at 100 U/kg/bid (5-6 animals vs 1 in other groups). Histopathology of testes indicated focal seminiferous epithelial atrophy with vacuolation of sertoli cells (trace-minimal) in 1, 1, 3, 4, and 7 males at 0, 5, 25, 100 of X14 and 100 of HM(ge) U/kg/bid resp. This was due to lower sperm count and motility.

Fertility and Early Embryonic Development in Female rats: Litter values: At 100 u/kg/bid, pre-implantation loss (9.9 vs 6.8 in controls) was higher. Post-implantation losses (12.9 vs 6.7 in controls) were higher also due to increased incidence of early embryonic death, leading to lower litter size and litter weight, Table 9.

**APPEARS THIS WAY
ON ORIGINAL**

Table 9. Litter values in rat segment I/II studies:

	Control	X14			HM(ge)
Dose (U/kg/bid)	0	5	25	100	100
# of Dams	24	23	23	23	24
# of Corpora Lutea	17.9	18.7	18.2	19.1	18.4
# of implantations	16.9	17.4	16.5	17	16.5
Pre-implantation loss (%)	6.8	5.9	8.2	9.9	10.6
# of In utero deaths (early+late)	1.2	1.3	1.3	2.2	2.6*
post-implantation loss (%)	6.7	7.4	9.1	12.9	16.1*
# of live fetuses	15.7	16.2	15.2	14.8	14.0*
Sex ratio (% male)	44.4	47.5	46.4	53.7	48.1
Litter weight (g)	57.5	59.1	55.8	54.2	53.0
Fetal weight (g)	3.7	3.7	3.6	3.6	3.8

*p<.05-0.01

Fetal examinations: Malformations and skeletal and visceral anomalies were observed with all doses of the drug. Small eyes/orbital sockets were observed, 3-4/23 litters were affected in all groups (at 5, 25, 100 and 100 U/kg/bid of X14 and HM(ge) resp), see appendix. Sponsor states that these effects on the fetus have been obtained in previous studies with insulin and are considered to be related to X14 or HM(ge)- induced reduction of maternal blood glucose, rather than the direct effect of the drug on embryofetal development, Table 10.

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Table 10. Malformations and skeletal and visceral anomalies in rat segment I/II toxicity study:

	Control	X14			HM(ge)
Dosage (U/kg/bid)	0	5	25	100	100
Visceral/Skeletal Malformations (FA/LA)	1/1	4/3	4/4	11/5	7/6
Litters examined	24	23	23	23	24
Fetuses examined	376	372	350	341	335
Cranial:					
Small orbital socket	0/0	2/2	3/3	4/3	4/4
Retinal fold	0	0	0	0	1/1
Thoracic					
Interventricular septal defects	0	0	0	2/1	0
Diaphragmaic hernia	0	0	0	1/1	1/1
scoliosis	0	0	0	2/2	0
Lumbar/Abdominal					
incomplete inferior vena cava	0	0	0	2/1	0
Visceral anomalies (FA/LA)	9/8	17/14	22/14	22/15	17/13
Litters examined	24	23	23	23	24
Fetuses examined	189	183	177	165	161
Cranial:					
Dilated lateral ventricle	0/0	0/0	0/0	0/0	1/1
small eye	0	0	1/1	1/1	3/2
Thoracic					
Interventricular septal defects	0	1/1	0	0	2/2
Lumbar/Abdominal					
Thin diaphragm with protrusion liver	0	0	0	3/3	3/3

FA= Fetuses affected, LA= Litters affected

The results indicate that X14 given to male and female rats subcutaneously before the matings, during the matings, and to females on days 0-15 postcoitus, caused anabolic/hypoglycemic effects (all doses reduced blood glucose levels). Two animals died at 100 U/kg/bid due to hypoglycemia (with associated erosion of stomach wall), 25-100 U/kg/bid increased body weight gains (in males by 26-34% and females by 38-58%), food consumptions (by 10-15%), caused testicular changes (focal seminiferous epithelial atrophy with vacuolation of sertoli cells), effects on litters (including pre- and post implantation losses and skeletal and visceral abnormalities (mainly affecting the axial skeleton and eyes). The response in general was similar to that seen with HM(ge). The drug had no effects on the male and female fertility or general reproductive performance of animals. Sponsor states that all the effects, including effects on fetuses, are due to insulin-induced maternal hypoglycemia.

Study title: Segment II Teratology study. Effects of Subcutaneous X14 (Twice Daily) On Embryofetal Development in Rabbits (Study # 940305)

Study No: 940305

Site and testing facility, study initiation-completion: _____

11/15/94-10/21/96.

GLP compliance: Yes

QA- Reports Yes (X) No ():

Lot and batch numbers: X14 06694 _____, 06994 _____, HM(ge) 05994 _____, 06094 _____

METHODS:

Species/strain: New Zealand White female rabbits, \approx 17-26 weeks of age, 2.95-4.25 kg.

Doses employed: 0, 0.5, 1.5 and 5 U/kg bid (or total doses 0, 1, 3 and 10 U/kg/day)

Route of Administration: Subcutaneous, 4-hrs apart

Number of animals/sex/dosing group: 20

Study Design: This study examined the teratogenic effects of X14 in rabbits. Four groups of 20 mated female rabbits were given the drug (at 0.5, 1.5 and 5 U/kg/bid) or recombinant human insulin (HM(ge) at 0.5, 1.5 and 5 U/kg/bid for comparative purposes), subcutaneously twice daily (4-hrs apart), from days 6 to day 18 of gestation. The reason for selecting lower doses here was based on the preliminary study in pregnant rabbits where doses of 6.25, 12.5 and 25 U/kg/bid were administered, which was associated with increased embryofetal deaths (mainly abortions) at all dose levels with highest incidences at 25 U/kg/bid, therefore lower doses were chosen here. Control animals received the vehicle (0.16% phenol, 0.172% m-cresol and 1.60 %, and 0.19% zinc). Surviving females were sacrificed on day 29 PC and necropsied.

Parameters and endpoints evaluated:

Clinical signs : Daily

Body weights/food consumptions: Body weights and food consumptions were recorded on day 0 (post coitum, PC), 2, 6, 8, 10, 14, 19, 23 and day 29 of pregnancy.

Plasma Glucose and TK: On day 6 PC (the first day of treatment), at 0, 1, 4, 5 & 8 hrs. Satellite animals (4/sex/group) were used for TK studies (study # 960419). Plasma samples were assayed for insulin X14 and human insulin.

Terminal examination of Females: Females were sacrificed on day 29 of pregnancy. The ovaries and uteri were examined. The number of corpora lutea, number of implantation sites, number of live/dead fetuses, and number of pre- and post- implantation losses, and fetal/placental abnormalities were recorded. All fetuses were weighed, sexed and examined for external abnormalities. Fetal brains were examined for abnormalities (hydrocephaly, hydroencephaly), and fetuses were examined for skeletal malformations by the modified Dawson's technique.

Statistical evaluations: Analysis of variance followed by intergroup comparisons were performed on BW, food consumptions, litter data and fetal abnormalities.

Analysis of incidence of fetal malformations were also performed using a one-tailed permutation test.

RESULTS:

Mortality and Clinical signs: One control animal was killed (pretreatment) on day 2 PC, following a delivery of 3 dead fetuses, this was replaced with another control animal. One animal was killed on day 25 of pregnancy at 5 U/kg/bid dose of X14 (it had lost weight, it had signs of inappetance and reduced fecal output, it had minimal GI contents, this was a drug related death, and had a plasma glucose levels of 9.0 mmol/l). There were 2 deaths with HM(ge) at 0.5 (not drug related) and 1.5 (on day 7 PC, drug related) U/kg/bid. No other clinical signs were observed.

Body weight: In females, during days 6 to 18 the body weight gains increased at 1.5 and 5 U/kg/bid (these were 212, 221, 338* & 357* g resp in 4 groups of X14, $p < 0.05-0.01$). These increases generally persisted (compared to controls) till termination day 29. These were also observed with 0.5-5 U/kg/bid of HM(ge) (280*, 283*, 305* resp), but did not persist till day 29.

Food consumption: In females, food consumptions were increased during treatment on days 6-18 at mid-high doses (98, 115*, 122*, 103, 112 and 108 U/kg/bid of X14 and HM(ge) resp), but returned to control values after stopping the treatment with X14, but not with HM(ge) at 5 U/kg/bid.

Plasma Glucose Concentrations: There was a dose related reduction in glucose conc at 1 hr, which returned to normal at 4-hrs, except at 5 U/kg/bid of X14 or HM(ge). The recovery at this dose was noted at 8 hrs with X14 (7.3 vs 7.5 in controls), while it was slower with HM(ge). The reduction in blood glucose was higher with 5 U/kg/bid of HM(ge).

On day 6:	1-hr	4-hrs
with X14:	7.5, 5.4, 5.0, 4.6	7.7, 7.8, 8.0, 5.4
with HM(ge):	5.4, 5.1, 3.9	8.1, 7.4, 5.6

Litter values: At 5 U/kg/bid, there were 2 abortions. At this dose pre-implantation loss (17.6 vs 13.3 in controls) was higher with X14. However it was lower with HM(ge) (7.1 vs 13.3% in controls). Post-implantation losses (34.9 vs 17 in controls) were higher with X14 or HM(ge), due to increased incidence of early embryonic death, leading to lower litter size and litter weight with X14. Fetal weights were not significantly altered. Similarly with 5 U/kg/bid of HM(ge), increase in early embryonic deaths were observed with decrease in litter size and weight, however none of the animals had abortions, Table 11.

Table 11. Litter values in rabbit segment II study:

	Cont	X14			HM(ge)		
Dose (U/kg/bid)	0	0.5	1.5	5.0	0.5	1.5	5.0
# of Dams	18	17	19	17	17	18	20
# of Corpora Lutea	11.1	10.5	11.4	11.2	11.4	11.4	11.0
# of implantations	9.6	9.0	9.9	9.2	10.0	10.6	10.2
Pre-implantation loss (%)	13.3	14.7	12.9	17.6	13.2	6.7	7.1
# of In utero deaths (early+late)	1.5	0.9	1.5	3.3*	1.1	2.1	3.6*
post-implantation loss (%)	17.0	9.5	14.0	34.9*	10.2	19.0	33.7*
# of live fetuses	8.1	8.1	8.4	5.9*	8.9	8.4	6.7*
Sex ratio (% male)	53.6	52.7	48.8	43.6	53.4	46.7	45.5
Litter weight (g)	356	348	382	275*	397	356	302
Fetal weight (g)	46.4	43.6	46.2	48.3	44.9	42.6*	46.4

*p< 0.05-0.01

Fetal examinations: At all doses of X14, but more at 5 U/kg/bid, there was a high proportion of fetuses with skeletal abnormalities affecting the vertebrae, ribs and sternum, these were observed mostly in cervical and thoracic regions, but also in lumbar and caudal regions. These were also noted with 1.5-5 U/kg/bid of HM(ge), Table 12.

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Table 12. Skeletal and visceral abnormalities in segment II toxicity study in rabbits:

	Cont	X14			HM(ge)		
Dosage (U/kg/bid)	0	0.5	1.5	5	0.5	1.5	5
Visceral/Skeletal Abnormalities (FA/LA)							
Litters examined	18	17	19	17	17	18	20
Fetuses examined	145	138	160	100	152	152	133
Visceral: Thoracic							
Absent intermediate lung lobe	3/1	0/0	4/4	3/2	4/1	2/2	0/0
Lumbar/abdominal							
Gall bladder-small/bilobed/bifurcated	1/1	1/1	4/3	1/1	6/3	2/2	2/1
Abnormal lobation liver	0	1/1	2/2	2/1	3/3	2/2	0/0
Skeletal Abnormalities (FA/LA)	20/ 13	30/ 14	33/ 13	30/ 13	24/ 8	36/ 15	34/ 15
Rib abnormalities: Absent (FA)	1	1	2	4	0	0	3
Fused/connected	0	0	3	7	0	2	6
Sternum: Fused/connected centers	1	2	3	3	2	1	3
Vertebral: scoliosis	0	0	3	2	0	0	2
Total vertebral/rib/sternal defects	5/3	9/8	10/6	19/ 11	5/4	10/9	13/7
Reduced ossification:cervical	5	10	14	4	1	3	7
Thoracic:	3	2	2	7	0	0	2
sacrocaudal	0	1	1	1	0	0	0
Cranial: Sutural bones	1	3	4	3	1	2	3
Irregular ossification of cranial bones	1	4	2	2	1	5	5
Thoracic-irregular costal cartilage	0	1	2	0	1	0	1
One less thoracolumbar vertebra	1	2	0	2	1	1	3

FA= Fetuses affected, LA= Litters affected

Toxicokinetics (Study # 960419, test facility Novo Nordisk Park, Malov):

Highest insulin X14 levels were generally noted at 1 hr and 5 hrs. Dose linearity was generally noted.

The mean values for groups were (nM):

Groups	X14	HM(ge)
1-hr	1.39, 4.56, 12.98	0.85, 6.03, 23.63
5-hr	1.49, 4.93, 18.83	1.17, 4.38, 17.19

The results indicate that X14 given to female rabbits subcutaneously from day 7 to day 18 of gestation, at doses of 0. 0.5, 1.5 and 5.0 U/kg/bid, (or HM(ge) at 0.5, 1.5, and 5 U/kg/bid), caused anabolic/hypoglycemic effects (all doses reduced blood glucose levels). One animal died at 5 U/kg/bid of X14, due to hypoglycemia. Similarly one animal at 1.5 U/kg/bid of HM(ge) also died. Mid-high doses (1.5-5 U/kg/bid) of X14 or HM(ge) increased body weight gains (by

59-68% & by up to 44% resp), food consumptions (by 15-22%), caused effects on litters (including pre- and post implantation loss, abortions and skeletal abnormalities (mainly affecting the axial skeleton). Sponsor states that all the effects are due to insulin -induced hypoglycemia including on fetuses. The response in general was similar to that seen with HM(ge).

Study title: Segment III study. Effects of Subcutaneous X14 (Twice Daily) On Pre- and Post-natal Development in Rats (Study # 940304)

Study No: 940304

Site and testing facility, study initiation-completion: _____
11/18/94-10/18/96.

GLP compliance: Yes

QA- Reports Yes (X) No ():

Lot and batch numbers: X14 06694 _____, 06994 _____ 07194 _____
_____, HM(ge) 06294 _____

METHODS:

Species/strain: Sprague-Dawley rats (CrI:CD BR VAF/plus strain), females 8-10 weeks old, 174-249 g.

Doses employed: 0, 5, 25 and 100 U/kg/bid, 4 hrs apart (or total doses 0, 10, 50 and 200 U/kg/day)

Route of Administration: Subcutaneous

Number of animals/sex/dosing group: 28

Study Design: This study examined the effects of X14 on pre and post natal development in rats. Also a group was included to receive recombinant human insulin HM(ge) at 100 U/kg/bid (total dose 200 U/kg/day) for comparative purposes.

Five groups of 28 mated female rats were given the three doses of the drug (at 0, 5

25, and 100 U/kg/bid), and the fifth group received HM(ge) subcutaneously, twice daily (4 hrs apart), from days 6 post coitum to day 20 of lactation (post partum), treatment for 1 more day continued after day 20 post coitum after the birth of young. Control animals received the vehicle (0.16% phenol, 0.172% m-cresol and 1.60 %, and 0.19% zinc). All adult females were allowed to litter and rear their young. At weaning offspring were selected and kept untreated for post weaning development (e.g sexual maturation, performance, behavior, and reproductive capacity). Excess females and pups were sacrificed shortly day 21 post partum and examined externally and internally for abnormalities.

Parameters and endpoints evaluated:

Clinical signs : Daily

Body weights/food consumptions: Body weights and food consumptions were recorded on day 0 (post coitum), 2, 4, 6, 8, 10, 14, 16, 18 and day 20 of pregnancy, and on day 0 of parturition and on days 1, 7, 14 and 21 post partum.

Water consumption: daily up to day 20 PC and again beginning day 1 post partum.

Plasma Glucose: On day 6 PC (the first day of treatment), at 0, 1 and 4 hrs after the first dose.

F0 Generation: Duration of pregnancy was calculated for adults (as the time between mating and the day on which pups were first seen). At delivery, the pups were recorded, examined for malformations, sexed and weighed on days 4, 8, 12, 16 and 21 PP and examined for clinical signs. Dead young were subjected to autopsy. Pups were examined for surface righting reflex, auditory startle reflex (for sensory and motor coordinating systems from day 1 and 11 resp), air righting reflex and pupils reflex (for auditory and visual functioning resp from day 14 and on day 20 resp). After day 21 PP, 24 male and female pups/group were used to form F1 adult generation, the rest were sacrificed and received the terminal examination, the uteri were visually examined for implantation sites.

F1 Generation animals: These were observed for clinical signs, body weights, sexual maturation, developmental/behavioral examination and accelerating rotarod test. When these rats were 84 days old, 1 male and 1 female from the same dose group were mated. F1 dams were observed and weighed on days 0, 7, 14 and 21 days PP. The F1 dams, F2 pups and F2 litters were evaluated as described earlier. F1 adults were sacrificed and subjected to necropsy/internal-external examinations.

Statistical evaluations: Analysis of variance followed by intergroup comparisons were performed on BW, food and water consumptions, duration of pregnancy, litter data, pre weaning and post weaning development data, and sexual maturation data.

RESULTS:

Mortality and Clinical signs: Three animals died at 25, and three at 100 U/kg/bid dose of X14 (these were deaths due to hypoglycemia). There were 8 deaths with HM(ge) at 100 U/kg/bid. Most of these deaths were associated with hypoglycemic episodes in the peri or post natal period, occurring on the same day or up to 5 days later. Therefore treatment was withheld after day 20, until day 1 post partum.

Body weight: In females, during days 8 to 14, the body weight gains increased at 100 U/kg/bid of X14 or HM(ge) (on day 14 these were 50.3, 53.9, 50.2, 55.3*, 58.4* g resp, *p<0.05-0.01). These increases generally persisted (compared to controls) till termination day i.e. at birth. These were also observed with HM(ge), but by end of lactation, these had returned to control levels.

Food and water consumption: In females, food/water consumptions were increased during treatment on days 6 to the first week of lactation at high doses of X14 and HM(ge), but food consumption returned to control values during the

last two weeks of lactation with both X14 and HM(ge). Water intake was also increased at 25 U/kg/bid

Plasma Glucose Concentrations: There was a reduction in glucose conc at 1 hr, which returned to normal at 4-hrs with 5 or 25 U/kg/bid of X14. However at 100 U/kg/bid at X14 or with HM(ge), the low glucose conc persisted.

On day 6 post coitum:	1-hr	4-hrs
with X14 and HM(ge):	9.5, 5.2, 4.5, 5.2, 5.0	9.4, 9.5, 9.3, 5.1, 4.9

Duration of pregnancy: Duration of pregnancy was increased ($p < 0.05$) at 25 and 100 U/kg/bid of X14 or human insulin (15, 19, 20, 25 and 23 days at 0, 5, 25, 100 and 100 U/kg/bid of X14 and HM(ge) resp)

Fo generation animals: One female from control and at 25 U/kg/bid groups, gave birth to a litter of entirely dead pups, this was not observed at higher doses. At 25 and 100 U/kg/bid of the drug or human insulin, 3, 3, and 6 litters resp were killed following the death of mothers. A higher pup weight (10, 9.7, 10.4, 10.7* and 11.0* g on day 4 resp) and litter weight (120, 124.8, 132.3*, 136.9* and 137.1* g resp, $*p < 0.05-0.001$) was noted with no significant effect on implantation loss (24, 28, 23, 24, 22, litters with 2 or less) with the drug or HM(ge). Pre-weaning development was not significantly altered, and slight changes were due to gestational variations.

F1 generation animals: No mortalities were observed. Females and males derived from 100 U/kg/bid HM(ge) had lower body weights (males by 7 % and females by 5% at weeks 4-21), also there was an increase in the proportion of dams with a longer (23 day) duration of pregnancy (6, 4, 3, 7 and 16 resp). The age for attaining sexual maturity in F1 females derived from parents treated at 25 or 100 U/kg/bid of X14 was significantly lower compared to controls (33.3, 32.8, 32.6*, 32.4*, and 32.6* days resp). sponsor states that these changes were slight, and not of major toxicological significance. Again with the drug or HM(ge), higher pup weight (10, 9.8, 10.1, 10.4 and 11.5* g on day 4 resp) and litter weight (90.3, 101.6*, 105.1*, 100.2*, and 100.9* g resp, $*p < 0.05-0.001$) was noted, with a slightly higher implantation loss (21, 23, 23, 20, 18 litters with 2 or less). No effects on post weaning behavior was observed. A slightly higher incidence of pups with minor kidney changes (mainly increased pelvic dilatation or pale discoloration) in litters derived from females with 100 U/kg/bid HM(ge) was noted.

In conclusion, In a segment III study in rats, old process X14 was given subcutaneously, at doses of 5, 25 and 100 U/kg/bid, or HM(ge) 100 U/kg/bid from days 6 of pregnancy to day 21 of lactation period (from implantation to weaning). At 25-100 U/kg/bid of X14, the drug caused 3 deaths/group (vs there were 8 deaths with 100 U/kg/bid of HM(ge), all due to hypoglycemia. Reduction in plasma glucose was noted with X14 and human insulin, and at low-mid doses the

blood glucose recovery was seen by 4 hrs. However, at high dose (100 U/kg/bid), the animals showed persistent hypoglycemia with X14 or HM(ge) at 4 hrs. At 100 U/kg/bid of X14, increases in body weight gains (slightly higher increases in body weight were observed with HM(ge)) and food/water consumptions were noted. In F₀ animals, a significant increase in proportion of dams with longer duration of pregnancy (22/23 days) was observed at 25-100 U/kg/bid or human insulin. In F₀ offspring, pup weight was slightly increased on day 4 post partum. F₁ rats had lower body weight gain, an increase in proportion of dams with longer duration of pregnancy (23-day), a higher implantation loss in litter, higher pup weight gain on day 4 post partum, and a higher incidence of pups with minor kidney changes. Sponsor states that these effects are due to the maternal pharmacological response to the drug.

Overall Reproductive Toxicology Summary

Following reproductive toxicity studies are summarized here: segment I & II fertility/teratology studies in rats, segment II teratology studies in rabbits, segment III peri-postnatal study in rats.

In a combined segment I/II fertility and embryo-fetal development study in rats, animals were treated with X14 (old process drug) at doses of 5, 25 and 100 U/kg/bid, or with HM(ge) 100 U/kg/bid (recombinant human insulin for comparative purposes) subcutaneously. Females were given old process X14 or HM(ge) for 14 days prior to mating, throughout the mating, and from days 0 to day 15 postcoitus. Males were given the drug for 28 days prior to mating, during mating, until their necropsy. One hundred U/kg/bid of X14 and HM(ge) caused 2 and 1 deaths resp, due to hypoglycemia (with associated erosion of stomach wall). Plasma glucose values were decreased with all doses at 1 hr, but took more than 4 hrs to recover (with X14 or human insulin) at 100 U/kg/bid. At 25-100 U/kg/bid, increases in body weight gains (in males by 26-34% and females by 38-58%), and food consumptions (by 10-15%) were observed, along with testicular changes in males (focal seminiferous epithelial atrophy with vacuolation of sertoli cells), and effects on litters were noted (including pre- and post implantation losses, and skeletal and visceral abnormalities, mainly affecting the axial skeleton and eyes). All drug doses reduced blood glucose levels. However the drug had no effects on the male and female fertility or general reproductive performance of animals. The response in general was similar to that seen with HM(ge). Sponsor states that all these effects are due to insulin -induced hypoglycemia, including effects on fetuses.

In a segment II teratology study in rabbits, pregnant animals were treated with old process X14 drug at doses of 0.5, 1.5 and 5 U/kg/bid, or with HM(ge) 0.5, 1.5 and 5 U/kg/bid (recombinant human insulin for comparative purposes) subcutaneously from days 6 to day 18 of gestation. Females were sacrificed on day 29 PC and necropsied. At 5 U/kg/bid of X14 or 1.5 U/kg/bid of HM(ge), both

drugs caused 1 death each/group, due to hypoglycemia. Dose related reduction in plasma glucose was noted with both X14 and human insulin, and at low-mid doses the blood glucose recovery was seen by 4 hrs. However, at high dose (5 U/kg/bid) the animals showed persistent hypoglycemia with X14 at 4 hrs, but there was a partial recovery with human insulin. At 1.5-5 U/kg/bid of X14, increases in body weight gains (by 59-68% & by up to 44% resp), food consumptions (by 15-22%), effects on litters, including pre- and post implantation loss, abortions and skeletal abnormalities (mainly affecting the axial skeleton) were observed. The response in general with the drug was similar to that seen with HM(ge). Sponsor states that these teratogenic effects of the drug are due to insulin-induced hypoglycemia, and related to the direct or indirect pharmacological effect of the drug.

In a segment III teratology study in rats, pregnant animals (F_0) were treated with old process X14 at doses of 5, 25 and 100 U/kg/bid, or with HM(ge) 100 U/kg/bid (recombinant human insulin for comparative purposes) subcutaneously from days 6 post coitum to day 20 post partum (PP). In a proportion of F_1 females behavioral development and reproductive capacity was examined. At 25-100 U/kg/bid of X14, the drug caused 3 deaths/group (vs there were 8 deaths with 100 U/kg/bid of HM(ge)), all due to hypoglycemia. Reduction in plasma glucose was noted with X14 and human insulin, and at low-mid doses the blood glucose recovery was seen by 4 hrs. However, at high dose (50 U/kg/bid), the animals showed persistent hypoglycemia with X14 or HM(ge) at 4 hrs. At 100 U/kg/bid of X14, increases in body weight gains (slightly greater increases in body weight gains were observed with HM(ge)) and food/water consumptions were noted. A significant increase in proportion of dams with longer duration of pregnancy (22/23-day) was observed at 25-100 U/kg/bid. Newborn pups at 100 U/kg/bid of X14 or HM(ge) showed slightly increased weight gain on day 4 PP, which was normalized by weaning. F_1 rats had lower body weight gain, an increase in proportion of dams with longer duration of pregnancy (23-day), a higher implantation loss in litter, higher pup weight gain on day 4 PP, and a higher incidence of pups with minor kidney changes. Sponsor states that these effects are due to the maternal pharmacological response to the drug due to insulin-induced hypoglycemia.

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